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Low dose effects of ionizing radiation on normal tissue stem cells

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Abstract

In recent years, there has been growing evidence for the involvement of stem cells in cancer initiation. As a result of their long life span, stem cells may have an increased propensity to accumulate genetic damage relative to differentiated cells. Therefore, stem cells of normal tissues may be important targets for radiation-induced carcinogenesis.

Knowledge of the effects of ionizing radiation (IR) on normal stem cells and on the processes involved in carcinogenesis is very limited. The influence of high doses of IR (>5 Gy) on proliferation, cell cycle and induction of senescence has been demonstrated in stem cells. There have been limited studies of the effects of moderate (0.5 – 5 Gy) and low doses (<0.5 Gy) of IR on stem cells however, the effect of low dose IR (LD-IR) on normal stem cells as possible targets for radiation-induced carcinogenesis has not been studied in any depth. There may also be important parallels between stem cell responses and those of cancer stem cells, which may highlight potential key common mechanisms of their response and radiosensitivity.

This review will provide an overview of the current knowledge of radiation-induced effects on normal stem cells, with particular focus on low and moderate doses of IR.

Key Words: Normal stem cells, irradiation, low dose, carcinogenesis.

Abbreviations

APE1: Apurinic endonuclease; ATM: ataxia telangiectasia mutated; BER: base excision repair; CSC: cancer stem cell; DNA DSB: DNA double strand break; ESC: Embryonic stem cell; EMT: Epithelial-to-Mesenchymal Transition; HD-IR: high doses of ionizing radiation; HR: homologous recombination; HSC: hematopoietic stem cell;

HPC: hematopoietic progenitor cells; iPSC: induced pluripotent stem cell(s); IR: ionizing radiation; LD-IR: low doses of ionizing radiation; NHEJ: non-homologous end joining; SC: stem cell(s); Sv: sievert; γ -H2AX: phospho-serine 139-histone variant 2AX.

1. Introduction

1.1 Conventional models of radiation-induced carcinogenesis

There is extensive evidence from animal and human exposures describing the risk of many cancer types, following acute radiation exposures [1;2]. The epidemiological data from the Atomic Bomb survivor cohort collected over 60 years supports a linear dose response relationship for intermediate doses, however for low dose exposures the evidence is less reliable due to lack of statistical power for cancer induction at low doses (<100 mSv) [3].

Conventional radiobiological models assume that cellular responses to radiation occur as a result of direct damage to nuclear DNA by a radiation track (known as 'target theory'). A further assumption is that damage is proportional to the number of tracks (which is related to dose) and therefore any dose no matter how small, can result in potentially mutagenic DNA damage.

These assumptions along with the epidemiology data for intermediate doses underpin the most frequently employed model for estimating radiation risk, the Linear No Threshold (LNT) model. This model only accounts for direct irradiation of cell nuclei. Therefore based on the LNT model, for all doses <1.5 Gy, the dose-response curve for excess cancer risk is linear. This is a conservative model that assumes any dose confers an excess cancer risk. In the low dose region this model is also supported by studies of *in utero* exposures in the order of 10 mGy that showed an increase in childhood cancers in exposed individuals [3].

There has been extensive debate concerning the suitability of this model for doses below 100 mSv and experimental studies in that dose region have provided evidence for a non-linear dose-response curve. This may impact on risk estimations after low dose occupational or medical exposures.

1.2 The new paradigm in radiation biology

Evidence from *in vitro* and *in vivo* studies in the last two decades has highlighted several issues that are not considered by conventional radiation carcinogenesis theories [4;5]. Firstly, the precise initiation event is difficult to pin point for radiation and is generally observed to be a stochastic process.

Secondly, a cancer outcome following radiation is most likely affected by the microenvironment, signalling between irradiated and non-irradiated cells and inflammatory responses. Finally, controversial ‘abscopal effects’ have been observed *in vivo* at sites distant from the irradiated area. These issues highlight the fact that mutation and subsequent cancer development cannot be explained by direct energy deposition in DNA only.

Low dose and targeted radiation studies have identified cellular phenomena that do not fit the traditional model as they elicit responses in cells that were not directly traversed by radiation tracks. These phenomena include genomic instability and bystander effects. Genomic instability describes an increased frequency of mutations and chromosome aberrations in the progeny of irradiated cells [6-8]. Radiation induced bystander effect describes the response of unirradiated cells to the irradiation of their neighbours. Radiation induced bystander effects have been observed for a range of biological endpoints including: apoptosis [9], DNA damage and up regulation of proteins in the DNA damage response, [6;10;11], micronucleus

induction [12;13], cell proliferation [14], cell survival [15-17] and genomic instability [18;19].

These processes have been found to saturate at low doses and to have non-linear dose responses. They are also often cell and radiation type specific and their existence indicates the need for better understanding of the mechanisms involved in radiation carcinogenesis and the development of alternative models for this complex process. Some more recent papers have described models of radiation effects that incorporate bystander signalling [20;21;22;23;24;25;26].

1.3 Stem cells as the target for the initiation of radiation carcinogenesis

Stem cells are undifferentiated cells, possessing the potential for unlimited replication and differentiation to many cell types (pluripotency). Key to this is the ability of stem cells can undergo symmetrical or asymmetrical division. Whilst in the first case two copies of the original stem cells are formed; the second case results in one daughter progenitor cell and one undifferentiated stem cell. Thereby stem cells can both self-renew and produce daughter cells capable of differentiating into one or more types of mature cell. The decision to divide by either route is stringently regulated by endogenous signalling and exogenous micro-environmental factors [27]. Stem cell fate is influenced by multiple convergent signal-transduction pathways the outcome of which is ultimately controlled by cell/tissue type specific 'master' regulators [28;29;30]. Key players in the decision for self-renewal or differentiation are the JAK/STAT and Hedgehog pathways as well as members of the transforming growth factor beta (TGF- β) family. TGF- β has an important impact on processes such as proliferation, differentiation, regeneration and homeostasis [31]. In cancer, TGF- β has a tumour-suppressive effect on premalignant cells. However, in the later stages of

cancer, TGF- β promotes invasion because of its role in epithelial to mesenchymal transition [32]. This process is also influenced by epigenetic regulation [33].

In mammals, there are two types of normal stem cells: embryonic stem cells (ESCs), which are isolated from the inner cell mass of the blastocyst, and can differentiate to form all cells of the three main germ layers (pluripotent). The second type of normal stem cells are adult stem cells. They act as a repair mechanism replenishing mature cells at a rate dependent on the requirement of the specific organ. Adult stem cells are typically slow cycling cells and, in general, can only differentiate into the cell types found in the tissue of origin although there are exceptions to this via reprogramming. They are defined as being multipotent. As a result of their long life span adult stem cells are thought to have an increased propensity for the accumulation of genetic mutations.

Are stem cells involved in cancer initiation?

Traditionally the development of cancer has been described to occur in three steps – initiation, promotion and progression. Carcinogenesis is now understood to be a complex process that occurs in a multiple stages, which have not been understood in any depth [34]. However, the fact that exposure with ionizing radiation (IR) can induce cancer has been known for over a century [35]. In recent years there has been increasing evidence to indicate the involvement of stem cells in cancer initiation, progression and tumour maintenance. The development of cancer and the possibility that cancers could arise from stem or stem-like cells (Cancer stem cells (CSCs)) is not a new idea, in fact this was proposed in the 18th century [36;37]. However it was not possible until the mid-1990s to isolate stem cell-like populations from a human cancer [38]. A good overview of the milestones contributing to the understanding of normal and cancer stem cells, has been published by Nguyen and

co-workers [36]. As a result of the many investigations in this context, the 'Cancer Stem Cell' hypothesis was born [37;39;40]. This theory assumes that normal stem cells can be transformed into CSCs (Fig. 1) and progenitor cells can be modified into cancer progenitor cells, which are able to generate differentiated cells that make up the bulk of the tumour. The key question that remains for the radiation protection and radiation biology communities is, what role radiation exposure plays in transformation of stem cells to CSCs and if this modification can be triggered by low dose irradiation. To our knowledge no detailed studies have been conducted that address this question.

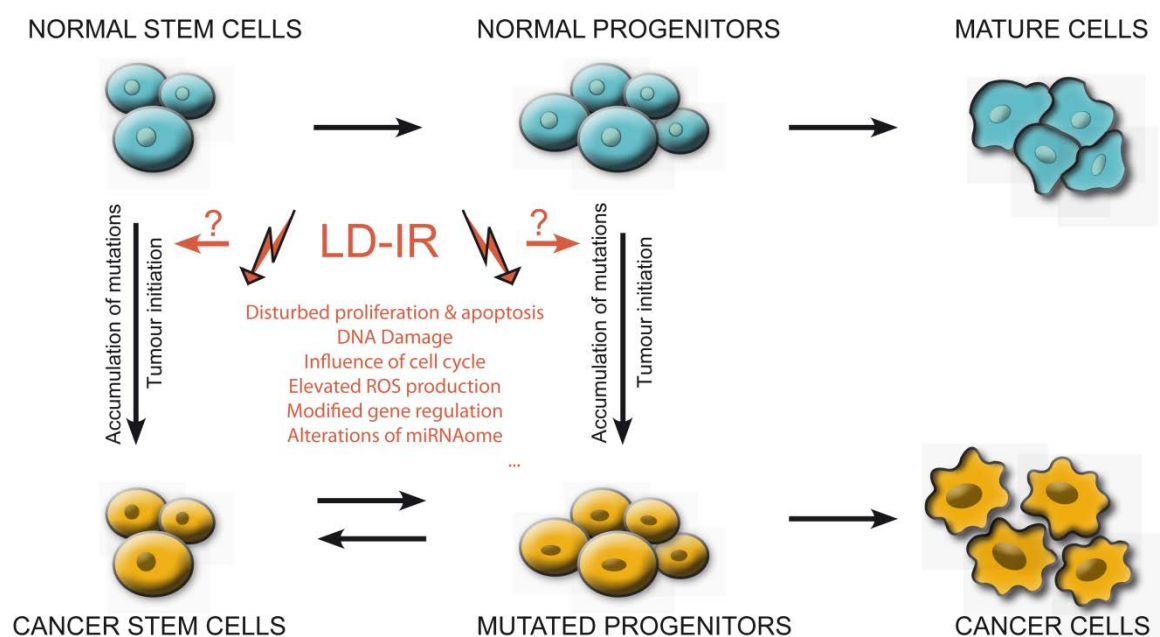


Figure 1: Proposed simplified model of theory for the origin of cancer stem cells; and the possible influence of low-dose irradiation (LD-IR).

The 'Cancer Stem Cell hypothesis' is supported by two main observations that originated in the 1970s [41], the first of which is the role of tumour heterogeneity. Although most tumours are thought to arise from a single transformed cell, solid

tumour masses are heterogeneous in nature suggesting the existence of a primitive cancer cell population capable of producing progeny from which diverse lineages can arise [42]. The second observation came from studies showing that a large number of cancer cells were required to grow and form a tumour [41;43;44]. In the CSC hypothesis it is postulated that a rare subpopulation of cells, CSCs, are responsible for tumor growth. Several xenotransplantation studies, involving serial dilution of pre-purified cells from human cancer cells in immunodeficient mice have shown that only CSCs were able to generate the tumours therefore supporting the CSC proposal [38;45-48].

Furthermore the 'Cancer Stem Cell hypothesis' suggests that, because of their long life time, stem cells may be the preferential targets of initial oncogenic mutations or accumulate additional mutations over a long period of time. Therefore stem cells with acquired mutations are thought to be the origin of many cancers [27;49-52].

In addition, besides immune suppression effects of cancer growth, the CSC hypothesis may explain cancer recurrence in patients that had been in remission for years or even decades after treatment [50], because conventional treatment may kill non-stem-like cancer cells, thereby decreasing the tumour bulk. CSCs remaining in the body are able to re-populate the tumor many years after the original therapy. This would also suggest that stem cells, or at least the so-called CSCs, are more resistant to conventional cancer therapeutics. Therefore, while the bulk of the differentiated cells within tumours are non-tumorigenic cancer cells with limited proliferative potential, and are relatively sensitive to treatments, the CSCs may survive treatments and retain their ability to self-renew and to regenerate the tumour mass. Consequently, therapies can diminish the tumor mass, but they will not cure the patient because the CSCs can cause tumor re-growth. However, the extent to which CSCs are present within individual tumours has been shown to be cancer site and

stage dependent [53]. The reported prevalence of CSCs may also be dependent on the assay used to detect CSCs [54;55]. However, there is now also evidence of a high degree of plasticity within tumour cells and therefore therapies that target mechanisms of de-differentiation may be very important [56;57]. Dedifferentiation of mature tumour cells to CSCs has been shown to be regulated by extracellular signaling from the tumour microenvironment through nitric oxide, transforming growth factor- α (TGF- α), transforming growth factor- β (TGF β), HGF and to activate WNT and NOTCH signaling pathways, thus 'switching on' stem cell signaling [58;59;60]. Hypoxia has also been shown in culture to play a role in increasing the CSC population by dedifferentiation [61].

During the last two decades CSCs have been identified in primary tumor isolates of many different cancer types [40;48]. Furthermore, many characteristic similarities have been observed between CSCs and normal stem cells [38;41;62]. Besides the involvement of stem cells in cancer initiation there is also growing evidence for the involvement of CSCs in cancer progression and metastasis [63;64]. Research, is aimed at refining treatment of many types of malignancies are now focused on targeting key pathways of CSCs so to more efficiently kill these resistant cells.

If stem cells are indeed the cells of origin of cancer, as much evidence to date suggests, then it is important to understand the mechanism of that transformation and critically the role played by low dose radiation exposures in initiating or stabilizing that process.

2. Radiation-induced effects on normal stem cells

The cancer stem cell-theory assumes that normal stem cells can be transformed into CSCs (Fig. 1). However it remains to be shown if radiation can trigger this transformation, and if this is the initiating step in radiation carcinogenesis. There have

been some studies showing how IR can influence normal stem cell fate (for detailed examples see, sections 2.2 – 2.4) but our understanding of the effect of IR on characteristics of stem cells per se as well as on the initiation and progression of cancer is very limited.

The following sections will give an overview of the current knowledge about radiation-induced effects on normal stem cells and the responses to different radiation doses in more detail. For the purpose of this review, classification of doses of ionizing radiation was used according to Kadhim et al. [5] as follows:

Very high – doses above 15 Gy

High – doses of 5 - 15 Gy

Medium – doses of 0.5 – 5 Gy

Low – doses of 0.05 - 0.5 Gy

Very low – doses below 0.05 Gy

2.1 Effects of high-dose (>5 Gy) irradiation on normal stem cells

During the last decade, the impact of radiation on different kinds of stem cell from normal tissue after exposure with high doses (>5 Gy) has been the topic of several studies. Following total body irradiation with 6.5 Gy murine hematopoietic stem cells (HSCs) were caused to senesce as a result of an increased level of ROS production [65;66]. Indications for increased induction of stress-induced premature senescence were also observed after *in vitro* irradiation of mesenchymal stem cells isolated from human bone marrow with <20 Gy. Additionally, irradiation resulted in reduced proliferation and p53 activation (after 20 Gy IR) [67], but no effect on cell viability or apoptosis, (measured by activation of caspases 3/7, 8 and 9) was observed in the

mesenchymal stem cells. In contrast to these results Filion et al., 2009 reported, in human ESCs, an induction of caspases 3 and 8 as well as expression of the anti-apoptotic protein Survivin after irradiation with 5 Gy [68]. Additionally, radiation induced DNA damage was detected as γ -H2AX foci, accompanied by phosphorylation of p53 at serine 15 and a G2 cell cycle arrest. An absence of a G1 arrest has been found in ESCs after DNA damage caused by 10 Gy doses of IR. Known pathways involved in DNA damage signaling and repair and cell cycle are thought to play a role in this response [69]. This is discussed further in section 3.

X-ray exposure of murine ESCs to 5-10 Gy induced a significant loss of heterozygosity of the *Aprt* gene [70], coding for the enzyme Adenine phosphoribosyltransferase, which is important in the purine nucleotide salvage pathway. It was observed, that the mutant frequency after X-ray treatment was 100-fold (5 Gy) higher than in differentiated cells, which has led to the suggestion that X-rays are a more potent mutagen for stem cells than for more differentiated cells. Contrary to point mutations that are observed in differentiated cells, after X-ray treatment of mouse ESCs, induction of mitotic recombination may be the main reason for the loss of heterozygosity.

2.2 Effects of moderate doses (0.5–5 Gy) irradiation on normal stem cells

Also for the moderate dose range of IR the effect on normal stem cells was investigated on the basis of standard radiobiological endpoints. In human ESCs a temporary G2/M (but not G1) arrest was observed 8 to 24 hours after irradiation with 2 Gy. This effect was shown to be dependent on ATM, a critical component of the DNA damage signalling pathway [71]. Wilson and co-workers (2010) performed various investigations of the effect of moderate IR on the normal human ESC cell line H9 [72]. Their experiments showed induction of apoptosis (measured by flow

cytometry) 48 hours after IR exposure. So an increase of apoptotic cell death after 2 and 4 Gy was clearly evident in comparison to unirradiated controls. Long-term study of the cells after exposure to 2 and 4 Gy revealed a temporary inhibition of cell proliferation that was distinctive only in the first week after exposure.

Beside well-known radiobiological endpoints, like DNA damage, cell cycle, proliferation or apoptosis additionally radiation-induced effects on miRNAome of normal stem cells as well as analyses on their gene expression and transcriptome were performed after moderate dose range of IR (0.5–5 Gy). There are reports revealing that moderate radiation doses can alter the miRNAome of human ESCs [73]. In human H1 and H9 ESC cell lines, 1 Gy irradiation leads to elevated miRNA control of various genes (shown by gene ontology analysis) such as those involved in positive regulation of cell differentiation, cell death and cell cycle, as well as activation of transcription. Further investigations of the H9 cell line have characterized the influence of 1 Gy on the human genome-wide transcriptome and shown that this was dependent on the time of analysis post treatment [74]. Early and late responses describe observed changes at 2 and 16 hours, respectively, after irradiation. The early radiation response involved up regulation of 30 genes that indicated a p53 dependent pro-apoptotic response. At the later time point of 16 hours after irradiation, a total of 354 genes were differentially expressed. The late response signature contained predominantly genes involved with pro-survival signaling pathways.

Transcriptomic analyses of the irradiated ESC cell line H9 by microarrays performed also by Wilson et al., 2010 [72] showed a higher degree of overlap between the samples of 2 and 4 Gy than with LD-IR samples (0.4 Gy) or with unirradiated controls. Increased co-clustering of genes between samples of the 2 and 4 Gy groups was observed compared to the unirradiated control (Global Pearson correlation of 95 % compared to 0 to 2 Gy: 87 %; 0 to 4 Gy: 89 %). Genes that have

been highlighted as being affected by moderate IR included those involved in cell death, p53 signalling, amino acid metabolism, cell morphology, molecular transport, cell cycle, TGF- β and Wnt signalling. Data from that study would suggest that IR does not significantly increase ESC differentiation.

The absence of an IR effect on differentiation of stem cells cannot be generalized because radiation-induced influences on stem cell differentiation were well described in other studies. For instance investigation of embryoid body formation for determination of the differentiation potential of induced pluripotent stem cells (iPSC) resulted in a dose dependent reduction of embryoid body diameter [75]. This effect was observed in embryoid bodies that were derived from irradiated mouse iPSCs after IR with 1, 2, 4 or 7.5 Gy X-rays, and were significant after 2 – 7.5 Gy. A significant radiation dose-dependent effect (from 1 – 7.5 Gy) was also shown in expression of the endoderm marker *Afp* which decreased in the embryoid bodies formed from irradiated iPSC in comparison to those formed from unirradiated cells. It is evident from the published observations that the effects of IR on differentiation are strongly dependent on the types of stem cell and endpoints studied.

The influence of moderate IR on gene expression in stem cells was also observed in other studies. Modifications of gene expression patterns were demonstrated in human HSCs after irradiation with 2 Gy. Up regulation of genes related to early haematopoiesis (FLI1; HOXB4; Tie-2), cytokine receptors (KIT; IL3RA), and oxidative stress (HO1; NQO1) has been described [76].

Furthermore, transcriptome analysis of human epidermal stem cells, 15 hours after 2 Gy exposure, by oligonucleotide microarray technology demonstrated an induction of a network of cytokines and growth factors as well as the repression of an apoptosis-involved gene network [77]. On the basis of clonogenic assays it was shown, that in contrast to the relatively radiosensitive progenitor cells the epidermal

stem cells were radioresistant. Therefore, radiosensitivity of normal stem cells appears to be definitely dependent on stem cell type and their tissue of origin. Besides the radioresistant properties (stem cells of skin or mammary gland), radiosensitive characteristics (brain stem cells) were also described (summarized in [27]). With regard to carcinogenesis, radioresistance of stem cells may provide increased possibilities for accumulation of mutations required for tumour initiation. Also, comparison studies of the radiosensitivity of normal stem cells and cancer stem cells have been performed and there are indications that normal stem cells are less radioresistant than their cancer counterpart [78].

Besides the effect of radiation on stem cells itself, the influence of radiation on the microenvironment may also play a crucial role in carcinogenesis. The effect of IR on stem cells in the niche at the hair follicle bulge region and their impact on radiation-induced hair greying has been investigated. A total-body irradiation of C57BL/6J mice with 5 Gy resulted in a stable induction of hair greying [79]. The authors suggested that the induction of differentiation of melanocyte stem cells after irradiation caused an abrogation of the stem cell self-renewal followed by hair depigmentation in the following hair cycles was responsible for the observed greying. Aoki et al, 2011 [80] also described that melanocyte stem cells, which were pre-damaged by irradiation, appeared to differentiate abnormally to ectopically pigmented melanocytes *in situ*. They furthermore observed *in vivo* an influence of the Kit signalling pathway on melanocyte stem cells in a radioprotective manner. Kit receptor signalling, an essential growth and differentiation pathway, plays a crucial role in regulating hair pigmentation of mammals [81]. In 2013 the same authors suggested that radiation-induced hair greying is probably caused by keratinocyte stem cells or keratinocytes rather than by melanocyte stem cells [82]. In both keratinocyte stem cells as well as

melanocyte stem cells, DNA DSBs after irradiation with 5 Gy were observed. But radiation exposed keratinocytes or keratinocyte stem cells suppressed the colony formation of melanocyte stem cells. The authors concluded therefore that irradiated keratinocytes or keratinocyte stem cells may serve as a niche factor for melanocyte stem cells.

2.3 Effects of low dose (<0.5 Gy) irradiation on normal stem cells

There is little evidence in the literature regarding the effects of LD-IR on stem cells. In H9 human ESC cell line, the induction of apoptosis 6 and 41 hours after treatment with low and moderate dose IR was investigated by Sokolov & Neumann [83]. Whereas moderate doses of 1 Gy resulted in significant apoptosis in the ESCs, low doses of 200 and 500 mGy produced no detectable apoptosis above the control level. A similar experiment with the H9 ESC cell line could also not detect apoptosis 48 hours after LD-IR with 400 mGy [72].

Whereas radiation exposure with low doses did not induced apoptosis, LD-IR was reported to cause modifications in gene and protein expression patterns. Genome-wide analysis of gene expression of H9 ESC cell line using microarrays showed a co-clustering of the 400 mGy sample with the unirradiated control (global Pearson correlation: 91 %; results of HD-IR see above) [72]. Similar to moderate doses (2 and 4 Gy) in the low dose range irradiated ESCs, IR affected expression of genes involved in cell death, p53 signalling, organ and embryonic development as well as cell cycle control. In C17.2 cells, immortalized mouse derived neural stem cells, LD-IR with 30 mGy (5 mGy/hour) was shown to cause an altered protein expression profile [84]. Both, up- and down-regulation were observed and the affected proteins were involved in neuronal development and function, neurodegeneration, cellular stress, apoptosis, cell cycle control and proliferation. Furthermore, authors reported

that doses of 10 and 30 mGy diminished differentiation of the immature neural C17.2 stem cells to glial cells.

Discontinuous dose dependencies after radiation in the low dose area were observed by Liang and co-workers (2011), who performed *in vitro* studies to investigate the influence of LD-IR on rat mesenchymal stem cells, isolated from the bone marrow of 6 to 8 week old male Wistar rats [85]. While treatment with 20 and 100 mGy had no effect on cell growth compared to unirradiated controls, exposures with doses of 50 and 75 mGy significantly stimulated the cell growth of the rat mesenchymal stem cells. The cause of the increase in cell growth has been attributed to activation of several members of the mitogen-activated protein kinases / extracellular-signal-regulated kinases (MAPK/ERK) signaling pathway in the rat mesenchymal stem cells after 75 mGy. The authors do not explain why doses of 50 and 75 mGy promoted proliferation but slightly lower doses (20 mGy) and higher doses 100 mGy had no influence. Potentially, non-linear dependencies such as those described for immune modulatory effects after LD-IR, play a role. There are many studies indicating that dose-response curves for LD-IR are non-linear, displaying discontinuous dose dependencies and that they reflect the hypersensitivity of cells to LD-IR not being predictable by extrapolation from the high dose IR response [86-88].

In addition to *in vitro* studies, *in vivo* experiments that focussed on the effect on stem cells of moderate and low doses of IR have also been performed. The impact of LD-IR on skin wound healing processes in response to repeated LD-IR (75 mGy X-ray, cumulative doses of 375, 600 and 825 mGy) has been investigated in diabetic rats [89]. Radiation induced stimulation of wound healing was connected to a time-dependent gradual increase in the number of bone marrow and circulating stem cells (cells which were positive for stem cell marker CD34⁺ and endothelial marker

CD31⁺). It can be postulated that LD-IR may have a stimulatory effect on proliferation of bone marrow stem cells.

Similarly, stimulation of proliferation of bone marrow hematopoietic progenitor cells (HPC) was observed in BALB/C mice 48 hours after exposure with LD-IR, which was most distinct after 75 mGy exposure [90]. Increased proliferation was accompanied by significant increases in HPC mobilization into the peripheral blood at 48 to 72 hours after LD-IR treatment. These results lead to the proposal that LD-IR may induce hematopoietic hormesis.

Radiation hormesis is a phenomenon in which a low dose of IR results in an adapted cellular response to subsequent exposures. In particular, there is evidence that this radio-adaptive effect may confer resistance to cells that have received a low 'priming' dose (reviewed in [91]), [92;93]. Whether or not a radioadaptive effect occurs in the case of normal stem cells is unclear. However, positive effects of LD-IR on stem cells *per se* and on processes dependent on stem cells, were also described by Wei and co-workers. On the basis of *in vitro* and *in vivo* studies with murine neural stem cells, a possible beneficial influence of LD-IR may exist in the neurogenesis of the mouse hippocampus. In contrast to HD-IR (3 Gy), LD-IR caused a stimulation of the Wnt/ β -catenin signaling pathway, which is assumed to be involved in regulation of proliferation and differentiation of neural stem cells as well as neurogenesis in the hippocampus. Elevated expression of Wnt1, Wnt3a, Wnt5a and β -catenin could be observed in the neural stem cells after IR with 300 mGy. Elevated proliferation and neuronal differentiation of neural stem cells after IR with 300 mGy were also detected. In addition, flow cytometry analyses revealed reduced apoptosis and improved cell survival of the neuronal stem cells [94].

Table 1 provides an overview of the studies investigating radiation responses (also in the range of LD-IR) of different types of normal tissue stem cells from human and

rodents. There are no reported studies in the literature investigating the effect of LD-IR on stem cells of normal tissue as possible targets for a radiation-induced carcinogenesis. The question of the involvement of LD-IR in the transformation of normal stem cells to cancer stem cells and subsequent carcinogenesis remains open.

In the last three sections there was given an overview of the current knowledge about radiation-induced effects on normal stem cells and the responses to different radiation doses. In fact, clear IR effects on normal stem cells were described (summarized in table 1), but a general conclusion with regard to dose dependency is still difficult. Because of the different dose ranges, unequal kinds of endpoints were investigated using several experimental designs. Additionally, a comparison of the IR effect on different types of stem cells, originating from diverse tissue as well as even different species, should be made carefully. Further studies using the same conditions, like the same stem cell types of origin, investigating identical endpoints using the same experimental design, will be necessary to fill the present gap.

3. Discussion and Outlook

Evidence suggests that for radiation carcinogenesis, stem or progenitor cells may be the cells from which the tumour originates. Irradiation with high doses, as well as moderate and low doses can influence stem cells at the single cell level, and more critically, processes that require stem cells, such as tissue development and maintenance. The effect of ionizing radiation on stem cells depends not only on radiation quality, dose and dose-rate [20;95] but also on endogenous factors such as the tissue of origin and microenvironment [96-98].

IR can influence stem cell fate by the induction of DNA damage, cell cycle arrest, senescence, cell death; these can occur through genetic and epigenetic changes that result in modified expression patterns.

Whether LD-IR can induce the transformation of normal stem cells into cancer stem cells is unknown and additionally the impact on carcinogenesis. Can LD-IR cause in normal stem cells deregulation of normal stem cell markers or induce expression of cancer stem cell markers?

Some insight into stem cell sensitivity to initiation by IR may be gained from research into the role of DNA repair pathways in stem versus more differentiated cells.

Mammalian cells have evolved extensive and robust mechanisms to recognize and respond to DNA damage, whether produced endogenously or exogenously. The mechanisms and the genetic defects that cause them to fail have been studied and extensively described for somatic cells [99], [100] and [101]. A robust DNA damage response is essential in stem cells in order to preserve their genomic integrity and that of their daughter cells if tissue homeostasis is to be maintained.

Despite the abundance of research on DNA damage responses, our knowledge of how different cell types respond to DNA damage is far from complete. Indeed the understanding of how DNA repair differs in stem compared to somatic cells is relatively recent and many questions remain. Some groups have investigated differences in DNA repair between stem and somatic cells and this may help to explain their response to IR. Most of that work has focused on DNA double strand break (DSB) repair in mouse and human embryonic stem cell models.

Research using mouse models that were generated to have defects in different pathways involved in DNA repair [102] have shown that HSCs accumulate spontaneous DNA damage with age. Concurrent with this increase in DNA damage accumulation the authors showed decreased efficiency of HSC stem-cell function,

and the accumulation of DNA DSBs was associated with increased cell death and decreased self-renewal. Although defects in all of the repair pathways that have been investigated have shown that this resulted in eventual weakening of long term self-renewal abilities of HSC *in vivo*, the most extreme response was observed when pathways required for DSB repair, particularly homologous recombination (HR) were affected. It seems likely that in HSCs the quiescent population can accumulate DNA damage over time without inducing apoptosis. Conversely the more rapidly proliferating progenitor cells are prone to apoptosis. Whether or not parallels can be drawn between quiescent HSCs and stem cells of other organs in this regard remains to be seen, however this suggests that stem cells are the more likely target for initiation than rapidly proliferating progenitors.

A study of murine ESCs also showed a dominance of HR over NHEJ when compared to somatic cells (80 % versus 20 %) [103;104]. As ESCs are proliferating rapidly, they are prone to endogenous DNA damage, however they have been generally observed to have lower mutation frequency relative to more differentiated cells [100].

When ESCs were grown in culture for prolonged passages they have been found to accumulate mutations. A mechanism involving the down-regulation of Apurinic Endonuclease 1 (APE1) and subsequent failure of BER has been described to explain this phenomenon in cultured human ESCs [105]. The authors reported that a decrease in the efficiency of the BER pathway meant that oxidative base damage was not converted by glycosylases to DNA DSBs [106;107] and therefore caused an accumulation of damage. The dominance of HR in ESCs is in contrast to somatic cells in which NHEJ is the dominant DNA DSB repair pathway and is possibly due to the large portion of time that these cells spend in S and G2 phase of the cell cycle when there is a template available for HR repair. Also, in contrast to somatic cells, IR induces predominantly G2 arrest in ESCs compared to G1 dominance in somatic

cells [71]. Additionally, the authors showed that in ESCs, γ H2AX foci, an established marker of DNA DSBs [108], colocalised with both RAD51 and Ku70, indicating that both HR and NHEJ were playing a role. This may explain the efficiency of DNA repair in those cells. It has been suggested that the conventional NHEJ pathway is less important in ESCs and that the higher fidelity alternative, XRCC4 dependent, NHEJ pathway is instead more prevalent. Interestingly, the DNA repair rate has been shown to increase with increased state of differentiation and this is thought to be due to the increasing role of NHEJ in DSB repair in more mature cells [109]. The alternative NHEJ pathway is still relatively poorly understood, and as this appears to have a role in the maintenance of stem cell genome integrity, this is an important avenue of future research.

How the stem cells of other tissue types respond to endogenous and exogenous sources of DNA damage (such as IR), the relationship to radiosensitivity and risk of carcinogenesis has not been well studied. From the limited examples available it is apparent and perhaps not surprising that sustained *in vitro* culture of stem cells modifies their response to stimuli. Data obtained in this way may not be representative or easily extrapolated to explain *in vivo* observations. As stem cells are localized in specialized stroma, or niche, and their response to stimuli (including IR) is conditioned also by many microenvironmental factors, results obtained through *in vitro* single cell systems may deviate significantly from *in vivo*. How IR affects the stem cell niche and how these modifications impact on the transformation of normal stem cells into cancer stem cells needs to be elucidated. It is acknowledged that *in vitro* and *in vivo* signalling factors are produced in response to IR that can induce responses in neighbouring unirradiated cells however little is known about how stem cells respond to these non-targeted effects [27;110;111]. Such inter cellular signalling

may involve modulation of immune and inflammatory responses may play an important role in the development and modulation of stem cell dependent cancer.

Until now, it is still unclear if IR exposure can induce transformation of normal stem cells into tumour stem cells and how important a role IR exposure plays in the initiation of carcinogenesis. Further investigations need to identify additional end points for characterization of stem cell dependent carcinogenesis. Further development is required in specialized 2D, 3D and *in vivo* models that can maintain stem cells and their progenitors in an environment that replicates the organ specific niche as essential tools that will enable extrapolation of results to the incorporation in and development of advanced models for human carcinogenesis.

Current projects

To answer the question of involvement of LD-IR in the transformation of normal stem cells to cancer stem cells and subsequent possible carcinogenesis two ongoing Euratom projects with experimental studies are in progress.

Both projects, EpiRadBio (Combining epidemiology and radiobiology to assess cancer risks in the breast, lung, thyroid and digestive tract after exposures to ionizing radiation with total doses in the order of 100 mSv or below; FP7-269553; 04/2011-03/2015) and ANDANTE (Multidisciplinary evaluation of the cancer risk from neutrons relative to photons using stem cells and the analysis of second malignant neoplasms following paediatric radiation therapy; FP7-295970; 01/2012-12/2015), address radiation-induced stem cell responses *in vitro* and *in vivo* respectively, with regard to the possible involvement of stem cells in carcinogenesis.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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